Research Article

Screening the Fruits of *Aegle marmelos* for Antibacterial, Anthelmintic and Cardiotonic Properties

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ABSTRACT

The present was study aimed at evaluating the antibacterial, anthelmintic and cardiotonic potentials of aqueous and ethanolic extracts of dried fruits of *Aegle marmelos.* Phytochemical tests on both extracts and powder material was done. The antibacterial activity was evaluated by cup diffusion method against both gram positive and gram negative bacteria. The ethanolic extracts showed more antibacterial activity than the aqueous extracts. Both the extracts having good activity against *Escherichia coli* (18±0.16mm and 19±0.68mm) compared to standard. The minimal inhibitory concentration of the each extract was determined both by broth culture method and agar cup diffusion methods. Ethanolic extract showed least minimal inhibitory concentration of 6.25µg/ml against *Escherichia coli.* The minimal inhibitory concentration values showed good correlation with experimental results. The ethanolic extracts showed more anthelmintic activity compared to the aqueous extracts. 50 mg/ml solution of ethanolic extract of *Aegle marmelos* showed significant activity (Time for paralysis: 28.32 ± 1.02min and Time for death: 40.16 ± 0.54min) compared to the standard. The Cardiotonic activity of the extracts was studied by using calcium free ringer's solution against isolated frog heart. The incremental doses of aqueous and ethanolic extracts were added which produces positive inotropic and negative chronotropic effects. Total experimental results supported the traditional use of *Aegle marmelos* for different pharmacological properties.

Keywords: *Aegle marmelos,* anthelmintic activity, antibacterial activity, cardiotonic activity, fruits

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INTRODUCTION

Green plants synthesize and preserve a variety of biochemical products, many of which are extractable and used as chemical feed stocks or as raw material for various scientific investigations. Many secondary metabolites of plant are commercially important and find use in a number of pharmaceutical compounds [1]. Phytochemicals with biological activity have had great utility as pharmaceuticals and pest management agents. Most of the medicines of previous centuries were of botanical origin. The ethno-botanical approach is still successfully used. For example, the antimalarial drug artemisinin from *Artemisia annua,* which is now being produced from plants in commercial quantities [2]. According to an all India ethno-biological survey carried out by the Ministry of Environment & Forests, Government of India, there are over 8000 species of plants being used by the people of India [3]. Nearly all 'wonder drugs' in use today are derived from natural products of about 120 plant derived drugs commonly in use in one or more countries, 74% were discovered as a result of chemical studies directed at the isolation of the active constituents of plants used in traditional medicine [4].

Many antibacterial agents are available in the market, which are developed by the great scientists. But still the microbes are challenging the scientists by developing the

resistance to the presently available drugs. Medicinal plants represent a rich source of antimicrobial agents. There is a need to identify new antimicrobials from plant origin to fight against the resistant microbes. Helminth infections are among the most widespread infections in humans, distressing a huge population of the world. The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases. Hence there is an increasing demand towards natural anthelmintics [5]. Cardiac disease will emerge as single largest Contributor to morbidity in India accounting for nearly one third of total deaths in near future [6]. Despite of the advancement of knowledge in understanding the basic pharmacology of cardio-active drugs glycosides still have its adverse effects in terms of toxication. It necessitates research for new nature based drugs which increase cardiac muscle contractility with a broad therapeutic index [7].

Aegle marmelos (L.) (Rutaceae) commonly known as bael or maredu (Telugu, India) growing wildly throughout deciduous forest of India, ascending to an altitude of 1,200 m in western Himalayas and also occurring in Andaman Islands. The fruits and leaves are valued in indigenous medicine**.** Poultice made of leaves is used for ophthalmia and ulcers. The leaves are used to reduce blood glucose level. Other actions like antibacterial, antifungal, antioxidant, antidiarrhoetic, pesticidal, antidote, anti-inflammatory properties, antispermatogenic has been reported [8]. The leaves are said to cause abortion and sterility in women. The bark is used as a fish poison in the Celebes. Tannin ingested frequently and in quantity over a long period of time, is antinutrient and carcinogenic [9]. Present study was aimed to investigate the cardiotonic, antibacterial and anthelmintic properties of *Aegle marmelos* fruit extract.

MATERIALS AND METHODS:

Plant materials and Extraction:

The ripen fruits of *Aegle marmelos* were collected from the forest areas of Maredumelli, East Godavari District, Andhra Pradesh. The pulp is separated from fruits and dried. After drying the pulp is powdered in hand pulverizer and passed through sieve no.: 20. The powder was collected and stored in an air tight container and stored at room temperature until the use. The powdered plant material was extracted by cold maceration method. The powder was macerated in ethanol and water separately for 24 hours. Then the powder was filtered through wattman filter paper no.40. The filtrate was distilled and evaporated on water bath up to a semisolid mass and air dried and kept in vaccum desicator and stored at room temperature.

Figure 1: EXPERIMENTAL PLANT - *AEGLE MARMELOS*

Collection of experimental cultures and animals:

Frog of *Rana tigrina* species were collected near fresh water ponds of korangi, East Godavari district, Andhra Pradesh and maintained in an animal house as per the norms of CPCSEA. Bacterial cultures of Gram- positive bacteria *Bacillus subtilis* (MTCC 441)*, Staphylococcus aureus* (MTCC 3160) and gram – negative bacteria *Escherichia coli* (MTCC 46) are used for screening. All the test strains were maintained on nutrient agar slopes and were sub cultured once in every two-week. The earth worms are collected from water logged soils near korangi, East Godavari district, Andhra Pradesh. They are washed with normal saline solution and stored in tyrode solution.

Preliminary phytochemical analysis:

Qualitative phytochemical analysis of the crude powder of the 12 plants collected was determined as follows: Tannins (200 mg plant material in 10 ml distilled water, filtered); a 2 ml filtrate + 2 ml $FeCl_3$, blue-black precipitate indicated the presence of Tannins. Alkaloids (200 mg plant material in 10 ml methanol, filtered); a 2 ml filtrate + 1% HCl + steam, 1 ml filtrate + 6 drops of Mayor's reagents /Wagner's reagent/Dragendroff reagent, creamish precipitate /brownish-red precipitate/orange precipitate indicated the presence of respective alkaloids. Saponins (frothing test: 0.5 ml filtrate + 5 ml distilled water); frothing persistence indicated presence of saponins. Cardiac glycosides (Keller-Kiliani test: 2 ml filtrate + 1 ml glacial acetic acid + $FeCl_3$ + conc. H_2SO_4); green-blue color indicated the presence of cardiac glycosides. Steroids (Liebermann-Burchard reaction: 200 mg plant material in 10 ml chloroform, filtered); a 2 ml filtrate + 2 ml acetic anhydride + conc. H_2SO_4. Blue-green ring indicated the presence of terpenoids. Flavonoids (200 mg plant material in 10 ml ethanol, filtered); a 2 ml filtrate + conc. HCl + magnesium ribbon pink-tomato red color indicated the presence of flavonoids [10].

Evaluation of antibacterial activity:

The antibacterial activity was done by cup diffusion method [11-14]. The cups are made by sterile cork borer (6mm) after solidification of the agar medium. The standard and test dilutions are made with DMSO as solvent. 100µg/ml solution of each extract was introduced into cups at sterile aseptic conditions. Ciprofloxacin (10µg/ml) solution was used as standard and DMSO was used as control. The plates are incubated in refrigerator for diffusion and then transferred to incubator and incubated at 37ºC for 18hrs. The zone of inhibition was measured and recorded.

The MIC of the each extract was determined both by broth culture method and agar diffusion methods. Two fold dilutions of the each extract were prepared and 1ml was introduced into 9ml of nutrient broth cultures and the growth was observed after 18hrs incubation. In agar diffusion method different concentrations of each extracts were introduced into cups and the zone of inhibition was calculated.

Evaluation of anthelmintic activity:

The anthelmintic evaluation of plant extracts was done by using Indian earth worms [15-20]. The different concentrations (25 mg/ml, 50 mg/ml and 100mg/ml) of crude ethanolic and aqueous extracts were prepared by triturating the sample in distilled water containing 15% tween80. 50ml formulation of standard drug piperazine citrate having three concentrations was prepared in 15% of tween80 and distilled water respectively. Suspension of distilled water and 15% tween80 was used as control.

Ten petridishes of equal size were taken and numbered. 50ml of formulation of different concentrations of both the extracts were placed in six petridishes. The different concentrations of piperazine citrate were placed in another three petridishes. Control was maintained on one petridish. All petridishes were placed at room temperature. The time of paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously and the time for death recorded after ascertaining that worms neither moved when shaken vigorously nor when they dipped in warm water at 50ºC.

Evaluation of cardiotonic activity:

Cardiotonic evaluation was done by isolated frog heart method [21-26]. Frogs were pithed and the heart was exposed. The inferior venacava was traced and cannulated for perfusing the heart with the frog ringer's solution. The basal cardiac contraction was recorded on kymograph after the administration of frog ringer's solution and tween80 (1%). The administration of tween80 was done to see that it did not contribute to the effect of extracts. The drugs and extracts are administered through cannula. The extracts were diluted with water and three dilutions are prepared- 0.25mg/ml, 0.5mg/ml and 1mg/ml. Digoxin (25µg/ml, 50µg/ml) is used as a standard drug and the responses of each dilution was noted on the kymograph. The amplitude of force of contraction and heart rate was recorded.

RESULTS AND DISCUSSION

Powder of fruit parts of plant was subjected to successive extraction by taking two solvent in increasing order of polarity i.e. ethanol, water and chemical tests on various extracts and powder material showed the presence of carbohydrate, gums and mucilages, proteins, alkaloids, cardiac glycosides, phytosterols, flavonoids, tannins and phenolic compounds, saponnins, fats and oils.

The ethanolic extracts showed antibacterial activity more than the aqueous extracts (**Figure 2**). Both the extracts showed less activity against *Bacillus subtilis*. It showed more activity on *Escherichia coli* compared to standard (**Table 1**). Both the extracts showed minimum inhibitory concentration (MIC) of 25µg/ml on *Bacillus subtilis* and 12.5µg/ml on *Staphylococcus aureus.* The minimum inhibitory concentrations of ethanolic and aqueous extracts were 6.25µg/ml and 12.5µg/ml respectively on *Escherichia coli* (**Table 2**). Totally the ethanolic and aqueous extracts of *Aegle marmelos* showed good anti bacterial activity. When the phytochemical screening was performed it was observed that the *Aegle marmelos* contains alkaloids, flavanoids, phenols and tannin which may either individual or in combination are responsible the antibacterial activity of *Aegle marmelos* fruit extracts.

Table 1: Anti Bacterial Activity of Ethanolic and Aqueous Extracts of Aegle Marmelos

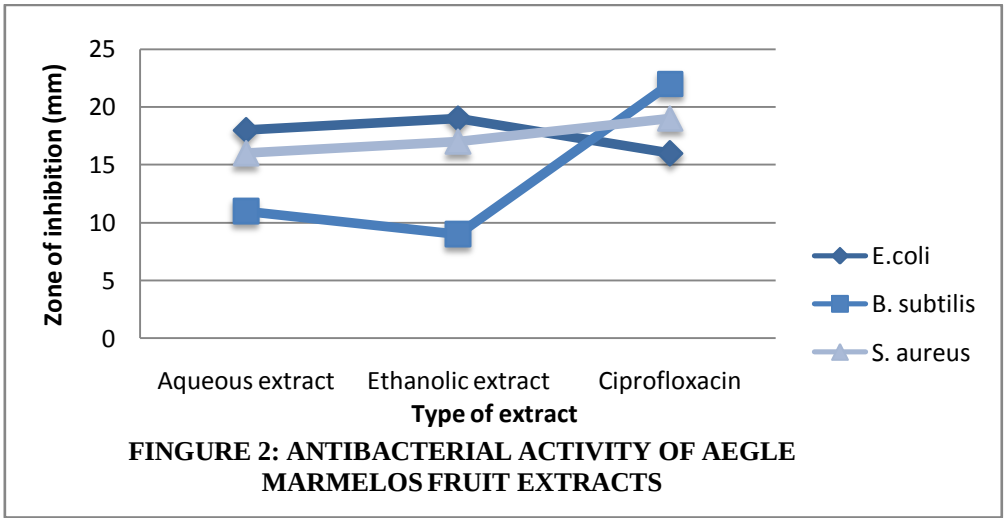
S. no	Type of extract	Diameter of zone of inhibition (mm)		
		Bacillus subtilis	***Staphylococcus aureus***	***Escherichia coli***
1.	Aqueous extract	11±0.20	16±0.68	18±0.16
2.	Ethanolic extract	09±0.24	17±0.44	19±0.68
3.	Ciprofloxacin (10 µg/ml)	22±0.82	19±0.82	16±0.46
4.	DMSO	--	--	--

Values are Mean ± SEM; n=3, (p>0.05).

Table 2: MIC of Ethanolic and Aqueous Extracts of Aegle Marmelos

S. no.	Bacteria	Aqueous extract (µg/ml)				Ethanolic extract (µg/ml)			
		50	25	12.5	6.25	50	25	12.5	6.25
1.	*Bacillus subtilis*	+	+	-	-	+	+	-	-
2.	*Staphylococcus aureus*	+	+	+	-	+	+	+	-
3.	*Escherichia coli*	+	+	+	-	+	+	+	+

(+): inhibition present *(-): inhibition absent*



FINGURE 2: ANTIBACTERIAL ACTIVITY OF AEGLE MARMELOS FRUIT EXTRACTS

The anthelmintic activity was performed on the Indian earth worms (*Pheretima posthuma*) and the results are reported (**Table 3**). The ethanolic extracts showed more activity compared to the aqueous extracts (**Figure 3 and 4**). 50 mg/ml solution of ethanolic extract of *Aegle marmelos* showed more activity compared to the standard. Both the extracts were found to poses anthelmintic activity. The phytochemical study shows the presence of flavonoids which are may be responsible for the anthelmintic activity.

Table 3: Anthelmintic Activity Ethanolic and Aqueous Extracts of Aegle Marmelos

S.no.	Type of extract	Concentration (mg/ml)	Time taken for paralysis (P) and death (D) of worms in min ± SEM	
			(P)	(D)
1.	Aqueous extract	25	34.23 ± 0.51	44.11 ± 0.36
		50	30.59 ± 1.26	41.24 ± 1.42
		100	20.42 ± 0.18	31.63 ± 1.16
2.	Ethanolic extract	25	35.16 ± 0.16	45.13 ± 1.01
		50	28.32 ± 1.02	40.16 ± 0.54
		100	20.13 ± 0.41	30.63 ± 0.88
3.	Piperazine citrate	25	33.30 ± 0.13	44.26 ± 0.45
		50	29.58 ± 1.43	40.10 ± 1.34
		100	19.60 ± 0.53	31.51 ± 1.19
4.	Control	--	--	--

Values are Mean ± SEM; n=3 worms in each group p< 0.05 is considered as significant when compared with standard drug.

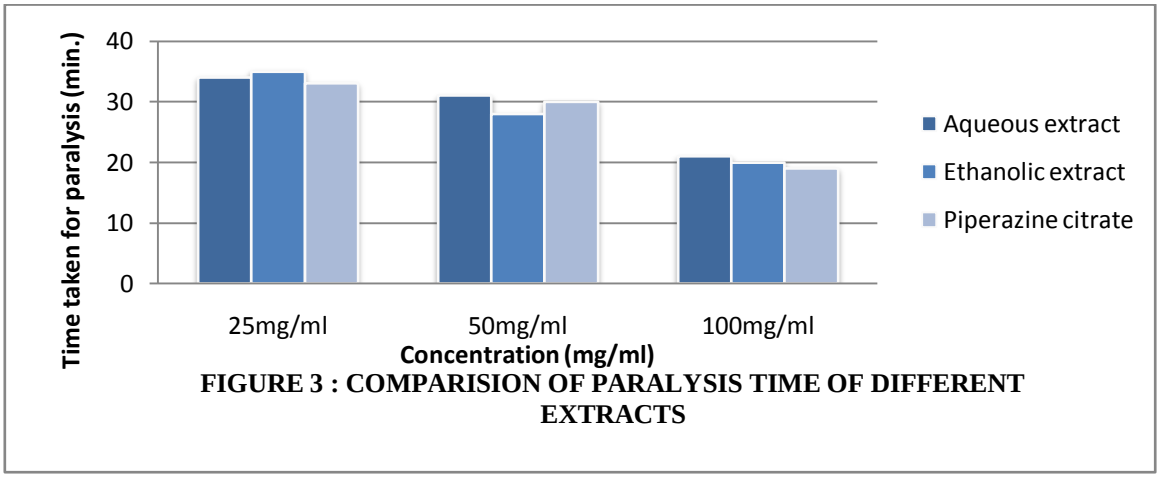


FIGURE 3 : COMPARISION OF PARALYSIS TIME OF DIFFERENT EXTRACTS

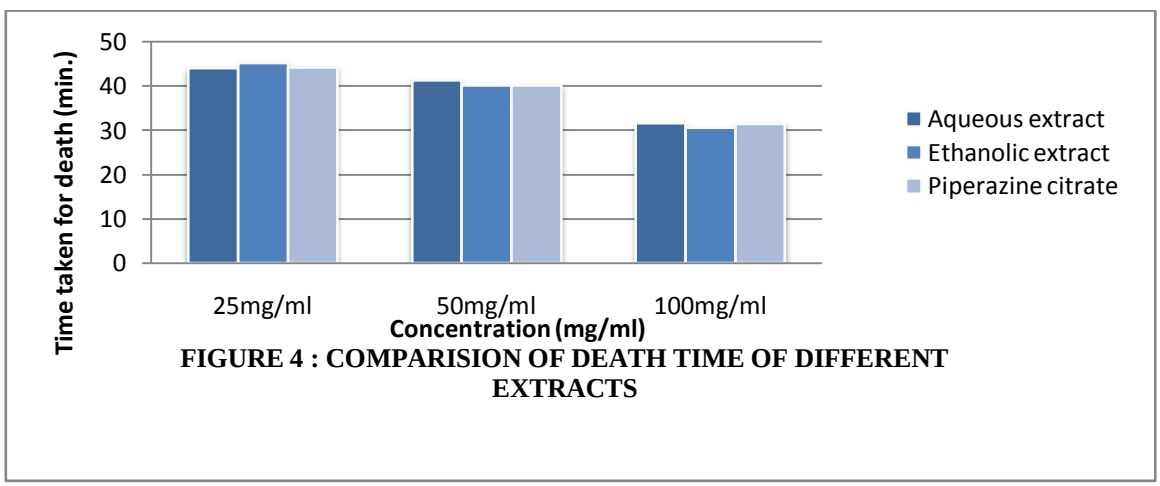


FIGURE 4 : COMPARISION OF DEATH TIME OF DIFFERENT EXTRACTS

The Cardiotonic activity of the extracts was studied by using calcium free ringer's solution and isolated frog heart perfusion technique. The incremental doses of aqueous and ethanolic extracts were added which produces positive inotropic and negative chronotropic effects. The two concentrations of digoxin (25 µg/ml, 50µg/ml) show positive inotropic and positive chronotropic effects on isolated frog heart. The results of Cardiotonic actions were reported in (**Table 4**). These effects showed the dose dependent increase in the activity (**Figure 5 and 6**). It is noted that *Aegle marmelos* extracts showed rapid onset of action.

The documented reports indicate that, the saponin glycosides and cardiac glycosides might be responsible for the positive inotropic effect [27], while the tannins and flavonoids provide free radical antioxidant activity and vascular strengthening [28]. The presence of saponins offers cardioprotective effects. The extracts under study contain all above mentioned phytoconstituents. Hence, it may be concluded that, the *Aegle marmelos* is endowed with significant positive inotropic effect with antioxidant, cardioprotective and vascular strengthening beneficial properties.

Table 4: Effect of Ethanolic and Aqueous Extracts from Fruits of Aegle Marmelos on Isolated Frog Heart Perfusion

S.no.	Concentration of extract or digoxin		Dose (ml)	HR (Heart rate Beats / min.)	HFC in mm. (Height of force of contraction)	Cardiac output: HR× HFC
1.	Aqueous extract	0.25mg/ml	Normal	54	5	270
			0.1	53	7	371
			0.2	51	8	408
			0.4	50	8.5	425
		0.50mg/ml	Normal	55	3	165
			0.1	54	5	270
			0.2	52	7	364
			0.4	50	10	500
		1.00mg/ml	Normal	49	6	294
			0.1	47	11	517
			0.2	45	15	675
			0.4	41	16	656
2.	Ethanolic extract	0.25mg/ml	Normal	52	6	312
			0.1	50	7.5	375
			0.2	48	8	384
			0.4	47	8.5	399.5
		0.50mg/ml	Normal	54	4	216
			0.1	52	6	312
			0.2	50	7	350
			0.4	48	9	432
		1.00mg/ml	Normal	48	6	288
			0.1	47	8	376
			0.2	46	10	460
			0.4	44	12	528
3.	Adrenaline	10 µg/ml	Normal	54	8	432
			0.1	57	9	513
			0.2	59	11	649
			0.4	61	12	732
4.	Digoxin	25 µg/ml	Normal	54	7	378
			0.1	56	8	448
			0.2	59	9	531
			0.4	61	12	732
		50 µg/ml	Normal	51	7	357
			0.1	53	12	636
			0.2	55	13	715
			0.4	58	13	754

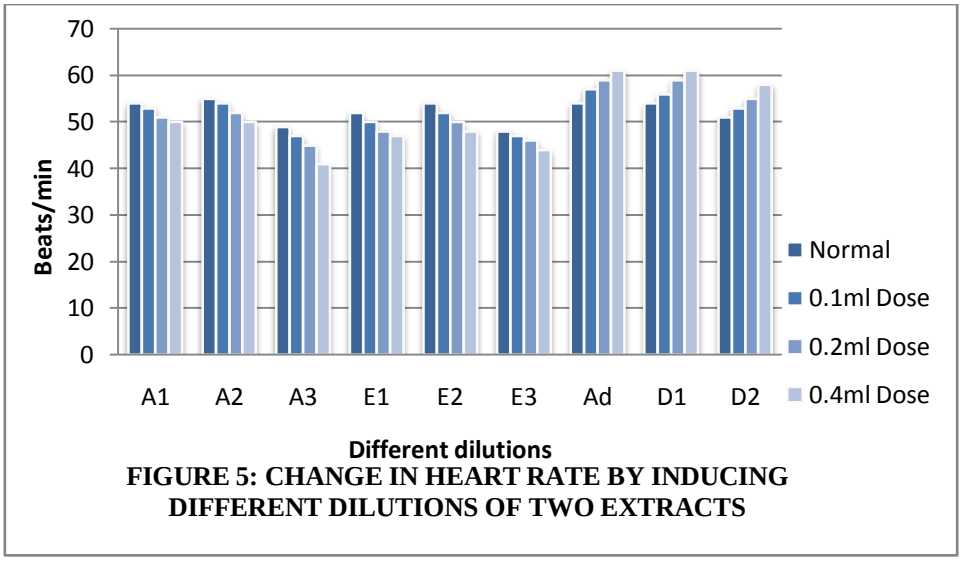


FIGURE 5: CHANGE IN HEART RATE BY INDUCING DIFFERENT DILUTIONS OF TWO EXTRACTS

A1: 0.25mg/ml aqueous extract; A2: 0.50mg/ml aqueous extract; A3: 1.00mg/ml aqueous extract; E1: 0.25mg/ml ethanolic extract; E2: 0.50mg/ml ethanolic extract; E3: 1.00mg/ml ethanolic extract; Ad: 10µg/ml solution of adrenaline; D1: 25µg/ml solution of digoxin; D2: 50µg/ml solution of digoxin.

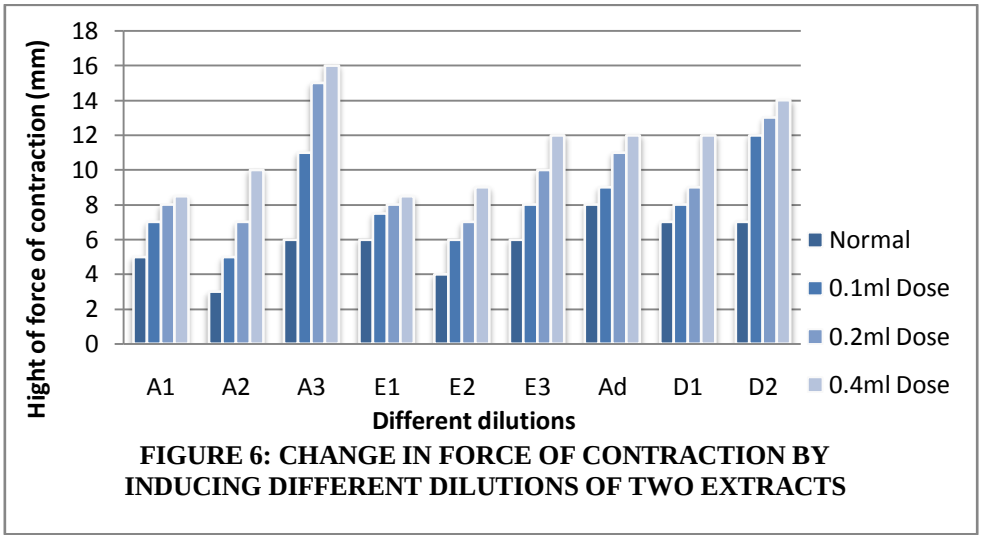


FIGURE 6: CHANGE IN FORCE OF CONTRACTION BY INDUCING DIFFERENT DILUTIONS OF TWO EXTRACTS

A1: 0.25mg/ml aqueous extract; A2: 0.50mg/ml aqueous extract; A3: 1.00mg/ml aqueous extract; E1: 0.25mg/ml ethanolic extract; E2: 0.50mg/ml ethanolic extract; E3: 1.00mg/ml ethanolic extract; Ad: 10µg/ml solution of adrenaline; D1: 25µg/ml solution of digoxin; D2: 50µg/ml solution of digoxin.

CONCLUSION

Based on the result in the study, it was concluded that extracts of *Aegle marmelos* fruit pulp were found to be having good cardiotonic, antibacterial and anthelmintic activities. Further studies are required to identify specific active principles of this plant for these significant pharmacological effects.

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